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Impact of Various *Brassica* species on the Developmental Response of *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae), its Extent of Damage, and Report of its Biocontrol Agent in District Rajouri of Pir Panjal Region of Himalaya

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Abstract

Cole crops are globally consumed as vegetables and are important sources of our dietary requirements, but they are infested by an array of pests, specifically the large white butterfly, *Pieris brassicae* (L). It is a critical global pest of cruciferous vegetable crops. We investigated the consequences of diverse cruciferous vegetables on the fitness of *P. brassicae* under laboratory conditions in 2021-22, to survive, develop, and reproduce under laboratory conditions, the extent of damage, and the report of the biocontrol agent, *Cotesia glomerata* (L). Under laboratory conditions, the effect of radish (*Raphanus sativus*), cabbage (*Brassica oleracea var. capitata*), and cauliflower (*Brassica oleracea var. botrytis*) on *P. brassicae* health were assessed by studying survival time, total development time, and mortality. Development time from hatching of eggs to adult emergence was longest on radish (32.6±0.84 days) and shortest on cabbage (25.0±1.41 days). In addition, the higher hatching percentage (96%), lower larval death, and higher yield of adults in cabbage indicated the fact of cabbage being the most appropriate food for *P. brassicae.* Life tables were dominant tools for analyzing and comprehending various aspects of an insect population's growth, survival, and reproduction. Furthermore, the crop loss was a function of pest population and the information on crop losses would serve as a guide for research programmes in crop improvement. The resistance to certain insecticides and the methods used to manage this insect pest on vegetable products in general required extensive research including biocontrol. We report the endoparasitoids *C. glomerata* (L) as a biocontrol agent against *P. brassicae* in district Rajouri which is the first record from the area which, therefore, warrants the essentiality of having a compact insect pest management plan that can relieve the dependency of destructive chemical control methods.

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Introduction

Among various vegetables, cole crops are significant crops widely cultivated in different climatic regions around the globe. The green leafy Brassicaceae vegetables have a wider array of micronutrients and sulphur-containing phytonutrients that promote health. They have a positive cardiovascular protective role against gastrointestinal tract cancer.[22] However, the qualitative and quantitative value of Brassicae vegetables is affected by different insect pests and is a very serious menace to the profitable cultivation of cruciferous vegetables. These crops are vulnerable to feeding damage from several chewing and sucking insect pests. Some pests attack the vegetables at root stages (e.g., wireworms), some attack the young seedlings (e.g. cutworms) while as some attack stems and foliage (e.g., caterpillars and beetles). Aphids suck the juices from the plants which results in an overall loss of plant vigor. Common insect pests on Brassicae in India includes cabbage butterflies: *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae), *Pieris rapae* Linnaeus (Lepidoptera: Pieridae), diamondback moth: *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), head caterpillar: *Crocidolomia binotalis* Zeller (Lepidoptera: Pyraustidae), Cabbage webworm: *Hellula undalis* Fabricius (Lepidoptera: Crambidae), aphids: *Lipaphis erysimi* Kaltenbach (Hemiptera: Aphididae), *Brevicoryne brassicae* Linnaeus (Hemiptera: Aphididae), and flea beetle: *Phyllotreta brassicae* Goeze (Coleoptera: Chrysomelidae).[8] Among these pests, *P. brassicae* is the key oligophagous insect pest of cole crops intended for human consumption. It has been found that it infested 83 species of Brassicaceae or Cruciferae and has attained the status of a major pest in various parts of India due to changes in ecosystems and habitats resulting in 30-40% yield loss in vegetables.[5, 21] It generates major losses compared to the expenses of man-made pesticides for its control.[14] *P. brassicae* is dispersed all over the world where Brassicaceae crops are cultivated and is employed as a model species in the field of insect pest biology.[27] In India, it spends winter in plains and migrates to mountainous regions during summer.[11]

The development of the insect, its survival, and its reproduction are affected by the type of host plant.[13,16,17,19] During larval development, host plant quality is a key determinant of both fecundity and fertility of adults.[3,4] The body size of herbivorous insects may vary depending on the host plant's quality, which can then impact life-history traits like fecundity, longevity, and survival.[3,25] Moreover, the growth and reproduction can be affected by the host plant and geographical sources.[36] Therefore, the discrepancies highlight the need for caution when comparing findings across regions.

The larvae devour the whole leaves of cauliflower, cabbage, and flower buds of broccoli and cause prevalent damage at all developing stages of cole crops, such as seedling, vegetative, and flowering stages[7, 34] thereby making the first line of defence- the pesticide application mandatory for cole crop production. Though pesticide application has revealed positive results by improving the quality and yield of food, their indiscriminate use causes serious consequences of bio-magnification and persistent nature.[26] Therefore, to control the insect pests in an eco-friendly manner, strategies encouraging biological control within the context of integrated pest management (IPM) are currently demanded. Studies on the fauna of parasitic insects in different regions are of immense significance for successful pest control as they destroy their hosts.[33] In the present study, we were concerned in investigating the consequences of diverse host plants on the fitness of *P. brassicae* in district Rajouri and enable farmers to utilise the most suitable control strategy including biocontrol towards crop management.

Materials and Methods

Study Area and Sampling

The Rajouri district is situated on the southerly foothills of the Pir Panjal Himalaya in the union territory of Jammu and Kashmir (India). It is located at 32°57′ to 33°34′ N latitude and 74°00′ to 74°48′ E longitude and has seven tehsils, which cover a total area of 2,630 km^2, out of which about half of the area is occupied by forests (1,267 km^2) . Its topography is predominately hilly, undulating, and is distinctive as it possesses three agro-climatic regions: subtropical, intermediate, and temperate, which favors the cultivation of some diverse crops at diverse and particular elevations. The zones have dissimilar land use patterns, cropping prototypes, and inhabitation. The areas for cole crop production were purposely and randomly selected in district Rajouri (J&K UT) (Fig. 1). The cole crops used were radish (*Raphanus sativus*), cabbage (*Brassica oleracea* var. *capitata*), and cauliflower (*Brassica oleracea* var. *botrytis*).

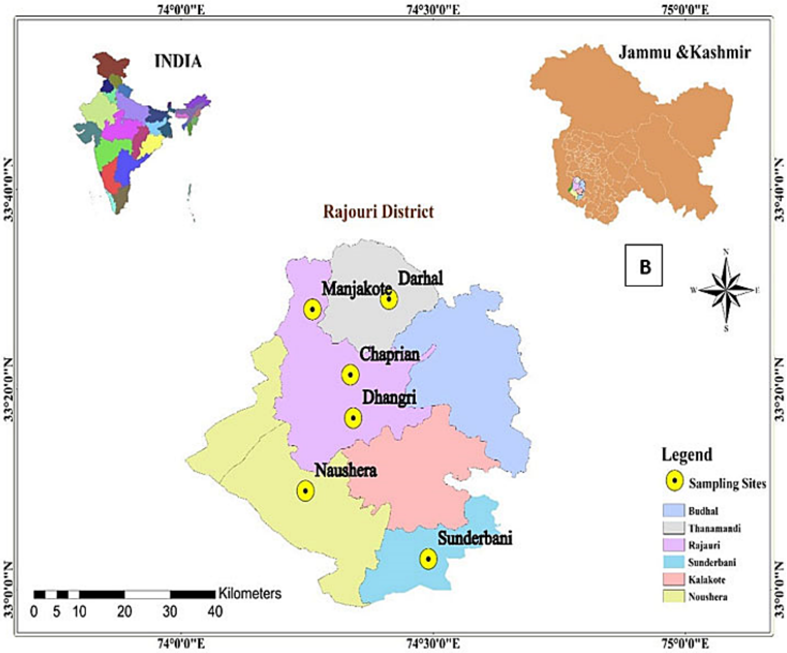

Fig. 1: Locations of different study areas in district Rajouri (J&K UT) India

Laboratory Investigation and Insect Rearing

Various *Brassica* crops were raised in the period 2021-22. The seeds were sown and watered on subsequent days. The seedlings were transplanted in a bed of size 5×5 m. The crop beds were monitored frequently in order to assess the infestation of the butterfly, *P. brassicae*. Before starting the experiment, field-collected insects were reared for a generation to culminate the effect of food reserves on the eggs and subsequent larvae. The leaf-laden eggs were carefully plucked from each Brassicaceae bed (stock culture) and taken to laboratory for mass rearing in the plastic jars (20×15 cm). Newly hatched larvae from their respective host plants were transferred to separate plastic jars (12×08 cm), covered with muslin cloth for continuous air supply, and fresh food was provided regularly until pupation. The adults emerged were given 10% sugar solution soaked in cotton balls. The progeny of laboratory-raised *P. brassicae* were used for developing a life table tailored to each level. The maximum temperature recorded during this experiment was 24.3 to 26.3 °C with a minimum temperature as 7.5 to 9.5 °C and the relative humidity recorded was between 78 to 84% in the morning and 32 to 45% in the evening.

Development, Survival, and Mortality

Leaf discs of 4 cm diameter were taken from cabbage, cauliflower, and radish and placed in three clean petri dishes (14×10 cm) covered with a moist filter paper. One freshly emerged larva was released on each leaf disc of the host plants, and each petri dish was chosen as a replicate. Larval food was changed each day and the duration of development of various instars of the larva, total developmental period of the larva, pupal period, and total developmental period of the insect were recorded. The data obtained were analyzed in MS Excel 2010 for calculation of average SD and SE.

Specific life tables of various phases were created in order to determine the effects of cole crops on *P. brassicae's* endurance and mortality stages. The data recorded from life tables from egg hatching to adult emergence were employed for computing different parameters like apparent mortality, survival fraction, mortality, survival ratio, indispensable mortality, and k values. It was computed following the protocol given by Ali and Rizwi.[2] The subsequent conventional bases were employed.

x = age of the insect

dx = insect died during the age interval, x

The parameters are briefly summarized as under

Apparent Mortality (100*qx*)

It gives the details of the numeral vanishing as a proportion of the number entering that phase and was calculated with the formula:

Apparent mortality (100qx) = (dx/lx) × 100

Where x is the age of the insect in days,
dx is the number of deaths during the age range x out of 100
Ix is the number of survivors at the start of each period x out of 100

Survival Fraction (Sx)

The survival fraction was used to calculate the stage-specific survival percentage (Sx) of each stage using data on apparent mortality. It is calculated by using the equation:

Sx of a particular stage = (*lx* of subsequent stage) / (*lx* of a particular stage)

Mortality Survival Ratio (MSR)

MSR is the population growth that would have occurred if the mortality in the relevant stage hadn't occurred. It is calculated as follows:

MSR of a particular stage = (Mortality in a particular stage) / *(lx* of subsequent stage)

Indispensable mortality (IM)

IM = Number of adults emerged × MSR of a particular stage

K-values

It is the primary element that drives the growth or decline in population from one age group to the next. The difference between the consecutive “log-lx” values is used to calculate the K value. By adding the k values for the various insect developmental phases, the overall generation mortality, K was determined according to Southwood, 1978.[31]

$$\mathbf{K = k_e + ki_1 + ki_2 + ki_3 + ki_4 + ki_5 + k_p}$$

where k_e, ki_1, ki_2, ki_3, ki_4, ki_5, and k_p are the k values at egg stage, first, second, third, fourth, fifth instars and, pupal stages respectively.

Biocontrol Agent and Insect Culture

The initial larvae of *P. brassicae* were collected from unsprayed cultivated cole crops (cabbage, cauliflower, and radish) during 2021-22 and were kept separately in rectangular plastic jars (25×20×15 cm) for rearing and for the recovery of parasitoids, if any. The jars were individually covered by a white cloth attached by rubber bands and fed with a fresh stock of food on subsequent days until pupation or parasitoids emergence, if any.

Ten *P. brassicae* of the first and second instar larvae were collected from the insect culture and placed in the centre of the respective host plants in ventilated plastic jars (10×15×10 cm) at room temperature. One disc of leaves was kept in each jar for each instar, and five adult male and female parasitoids were released in each plastic jar. Caterpillars were considered parasitized following Poelman et al (2014).[20] To record the pupal period, the newly formed cocoons were separated, placed on clean petri plates and observed every 12 hours for the emergence of the parasitoid. The number of days was recorded from parasitism beginning to adult appearance to compute the developmental time of the host caterpillars. The experiments were replicated five times. The data obtained were subjected to MS Excel 2010 software to calculate the average SE and SD.

Results

Life Cycle/ Development of *Pieris Brassicae*

Mating was usually observed in an end-to-end position. The female raised the wings by exposing its end abdomen, and the male mounted on the female’s back. The female butterfly laid eggs in masses, with the biggest mass comprising between 120 to 200 eggs, mostly on the lower surface of the leaves. A complete life cycle is shown in Fig. 2. The incubation period, larval period, and total developmental time in different host plants are shown in Table 1.

The developmental periods from egg hatching to pupa formation varied significantly amongst the host plants. The larvae reared on cabbage had a shorter total development time (28.6 days), while as those reared on cauliflower and radish have longer developmental time of 30.4 and 32.6 days respectively.

Distinct Phases of *P. brassicae* Survival and Mortality (Table 2, Fig. 3)

Table 1: Overview of *P. brassicae* developmental period (days) using various host plants in a laboratory

Stages	Cabbage		Radish		Cauliflower	
	Mean	SE	Mean	SE	Mean	SE
Incubation period	03.6	0.69	05.2	0.63	05.2	0.69
Larval period	19.2	1.22	25.2	1.07	22.3	0.67
Pupal stage	05.8	0.78	07.2	0.78	04.5	0.52
Total development period	28.6	1.17	32.6	0.84	30.4	1.42

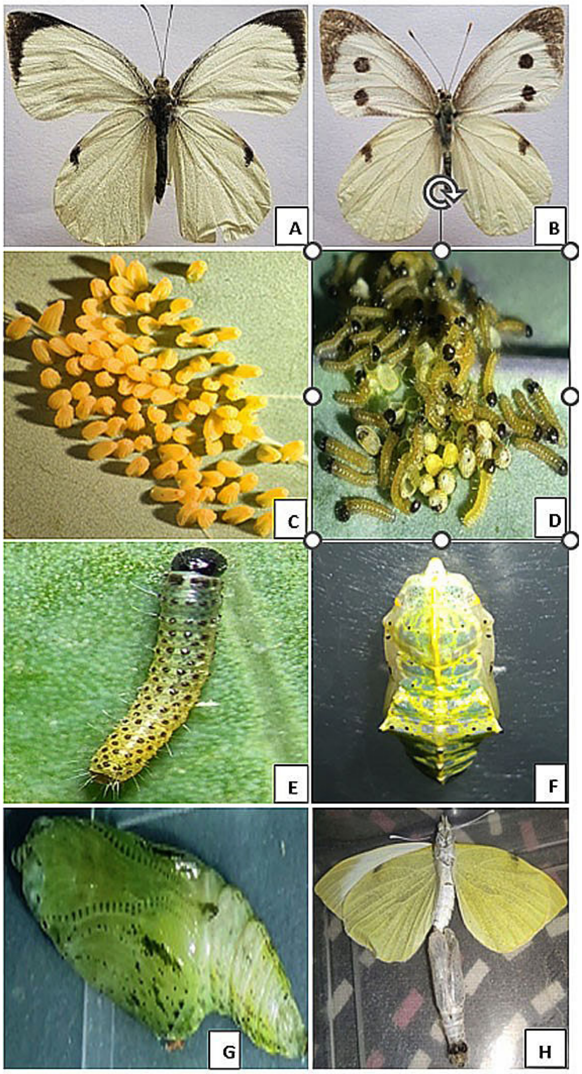

Fig. 2: Life cycle of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) (a, b) Male & Female (c) Eggs (d) Hatching (e) An instar larva (f) Pupa (g) Pre emergence (h) Emergence of adult

Table 2: Survival and mortality of various developmental phases of *P. brassicae* on diverse host plants under laboratory situation

Stages (x)	No of surviving at each stage (lx)	No of dying at each stage (dx)	Apparent mortality (100qx)	Survival fraction (sx)	Mortality/ Survival Ratio (MSR)	Indispensable mortality (MSR×no of adults emerged)	log 10	k value
Cabbage								
Incubation	100	04	4.00	0.96	0.04	2.84	2.00	0.02
I Instar	96	02	2.08	0.97	0.02	1.42	1.98	0.01
II Instar	94	05	5.31	0.94	0.05	3.55	1.97	0.02
III Instar	89	09	10.11	0.89	0.11	7.81	1.95	0.05
IV Instar	80	07	08.75	0.91	0.09	6.39	1.90	0.04
V Instar	73	01	01.36	0.98	0.01	0.71	1.86	0.00
Pupa	72	01	01.38	0.98	0.01	0.71	1.86	0.01
Adult	71	-	-	-	-	-	1.85	K=0.15
Radish								
Incubation	100	10	10	0.9	0.11	3.63	2.00	0.05
I Instar	90	07	7.78	0.92	0.08	2.64	1.95	0.03
II Instar	83	05	6.02	0.94	0.06	1.98	1.92	0.03
III Instar	78	09	11.54	0.88	0.13	4.29	1.89	0.05
IV Instar	69	15	21.74	0.78	0.28	9.24	1.84	0.11
V Instar	54	10	18.52	0.81	0.23	7.59	1.73	0.09
Pupa	44	11	25	0.75	0.33	10.89	1.64	0.12
Adult	33	-	-	-	-	-	1.52	K=0.48
Cauliflower								
Incubation	100	13	13	0.87	0.15	8.25	2.00	0.07
I Instar	87	05	5.75	0.94	0.06	3.3	1.93	0.02
II Instar	82	10	12.19	0.87	0.13	7.15	1.91	0.05
III Instar	72	08	11.11	0.88	0.12	6.6	1.86	0.05
IV Instar	64	04	6.25	0.93	0.06	3.3	1.81	0.03
V Instar	60	02	3.33	0.96	0.03	1.65	1.78	0.02
Pupa	58	03	5.17	0.94	0.05	2.75	1.76	0.02
Adult	55	-	-	-	-	-	1.74	K=0.26

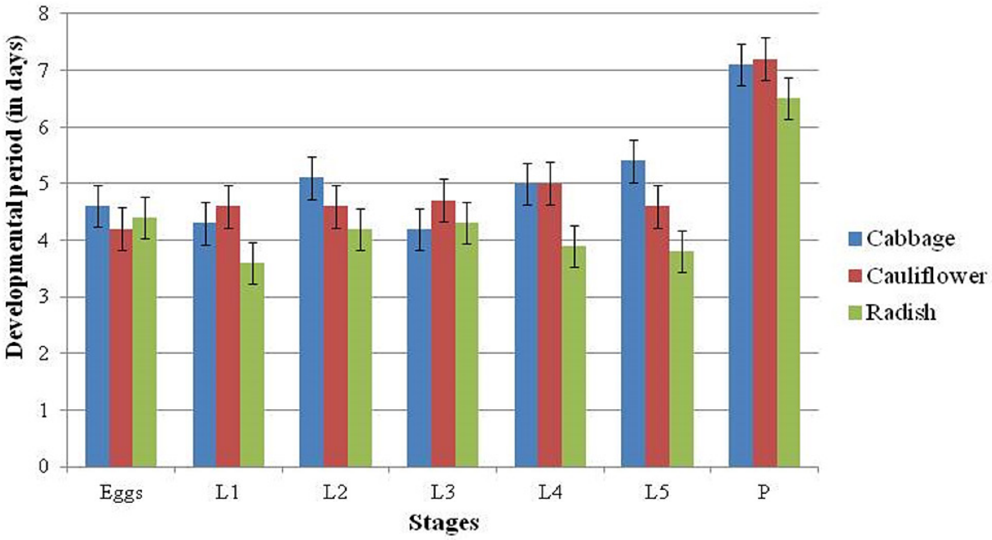

Fig. 3: Duration of various *Pieris brassicae* developmental phases on various host plants in a laboratory setting. L1 to L5 is the duration of larval instars, P is duration of pupae and the bars on the column represent the standard error

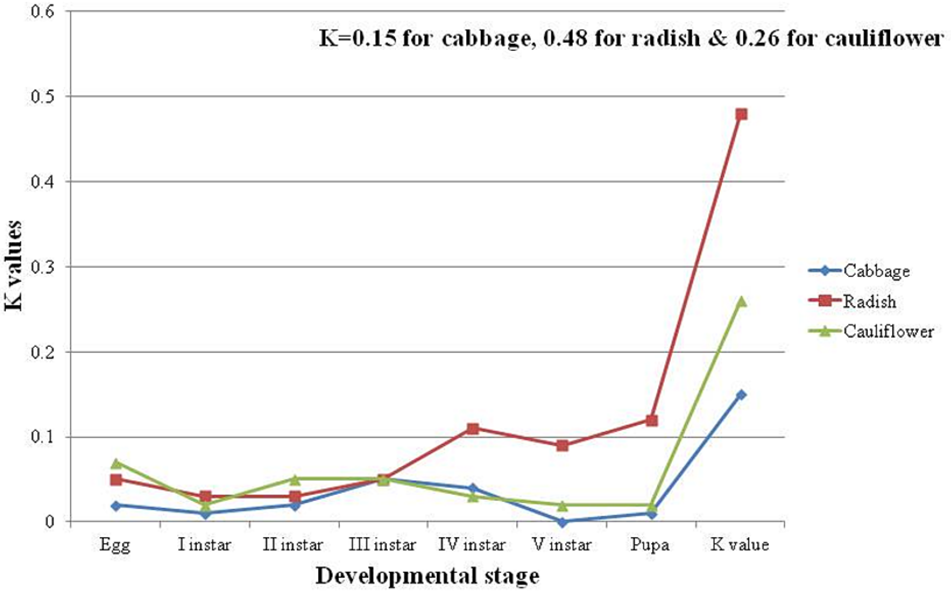

Fig. 4: K-values of *Pieris brassicae* at various developmental stages on diverse host plants under laboratory conditions. (K is total generation mortality)

Apparent Mortality

There was evidence of egg failure in all three host plants, with maximum egg mortality occurring on cauliflower (13%), followed by radish (10%) and cabbage (4%). However, larval mortality was observed highest in the second instar in radish (21.74%) and lowest in the fifth instar in cabbage (1.36%). Moreover, at the pupal stage, the highest mortality was observed in radish (25%) and the lowest in cabbage (1.38%).

Survival Fraction

From the observation, the maximum survival fraction at egg stage was found on cabbage (0.97) in the first instar, followed by cauliflower (0.96) in the fifth instar and the lowest on radish (0.75).

Mortality Survival Ratio

At the egg stage, the highest mortality survival ratio (MSR) was found on cauliflower (0.15), on cabbage (0.04). At various larval instars, radish had the highest MSR (0.28) in the fourth instar and the lowest MSR was found in cabbage (0.01) in the fifth instar. Moreover, at the pupal stage, the highest and lowest MSR was found on radish (0.33) and cabbage (0.01), respectively.

Indispensable Mortality

The highest and lowest indispensable mortality (IM) of eggs was found on cauliflower (8.25) and cabbage (2.84), respectively. At the stage of pupal development, radish had the highest IM (10.89), while cabbage had the lowest IM (0.71).

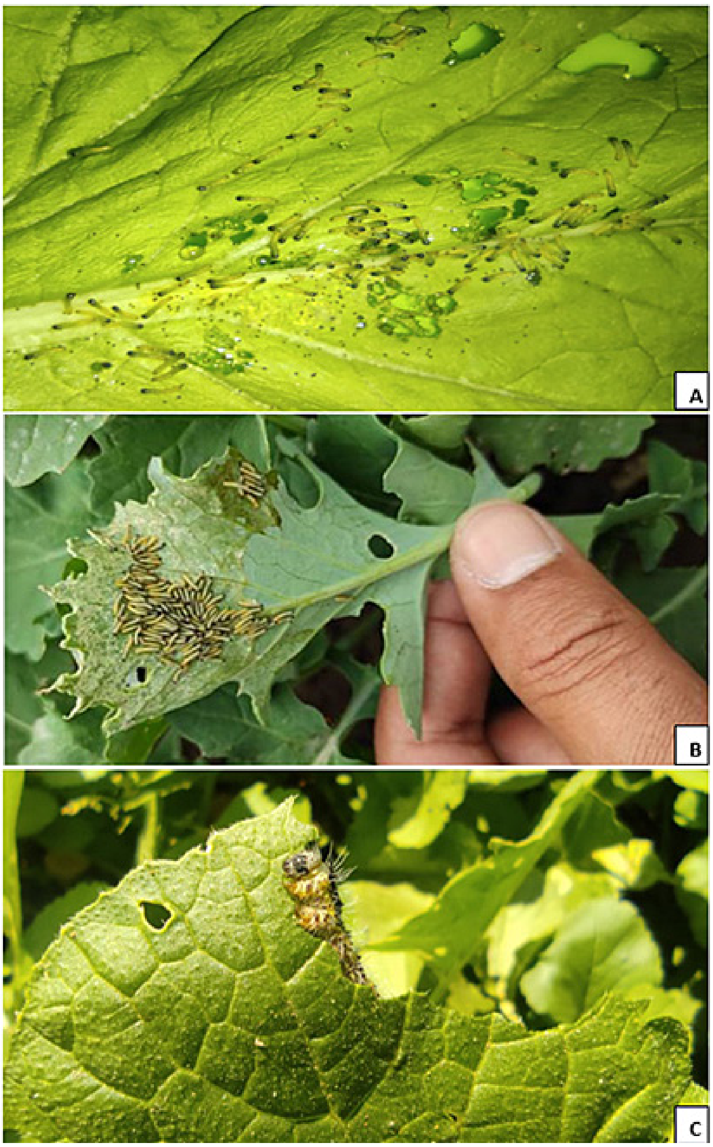

Fig. 5: (a) Hole formation, (b) scrapping of epidermal part of leaf, and (c) Leaf margin feeding by *Pieris brassicae* larva

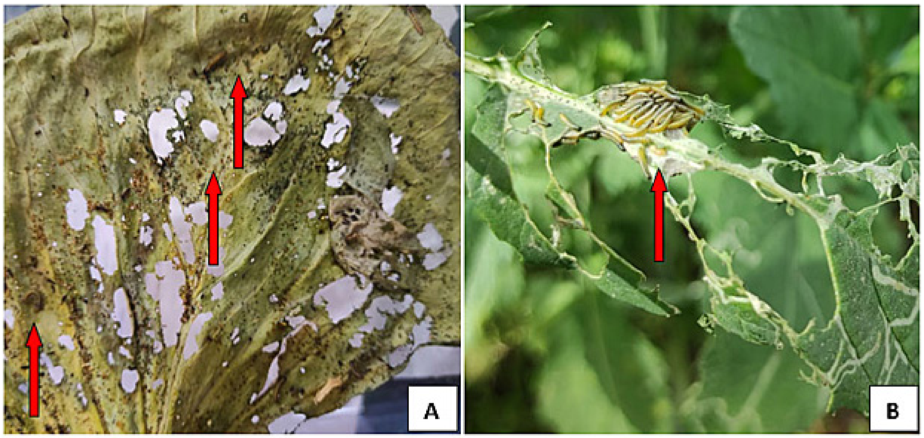

Fig. 6: Skeletization of cole crops by larvae of *Pieris brassicae* (a) Lab (b) Field

K Values

At the egg phase, cauliflower had the maximum k-value (0.07), while radish and cabbage had the K values of 0.05 and 0.02, respectively. Regarding the larval instars, the k-values were highest for radish (0.11) in the fourth instar and lowest for cabbage (0.00) in the fifth instar. Besides, the total generation mortality, K[31, 35] of *P. brassicae* was recorded lowest on cabbage (0.15) and highest on radish (0.48), followed by cauliflower (0.26); thereby, cabbage resulted the most suitable host for *P. brassicae* development (Fig. 4). Moreover, the highest yield of adults was obtained from cabbage (71) in contrast to radish (33) and cauliflower (55).

Extent of Damage

The caterpillars alone fed on leaves. The adult female butterflies only lay eggs and relied on visual cues to decide where to lay eggs. The plants used as oviposition sites typically contained glucosides. The caterpillars fed gregariously, and the initial caterpillars of the first instar stage just scraped the leaf surface and sometimes created circular irregular holes, but the grown-up caterpillars wandered from plant to plant and ate up the leaves in the middle of the leaf lamina and by the sides of the leaf margins, leaving intact the main veins, thereby resulting in heavy yield losses (Fig. 5, 6).

Parasitization and Behavior

Cultures of *C. glomerata* and *P. brassicae* were established from individuals collected from the agricultural fields. Laboratory rearing of field-collected larvae of *P. brassicae* revealed parasitism by a single wasp species, *Cotesia glomerata* Linnaeus (Hymenoptera: Braconidae), formerly known as *Apanteles glomeratus* Linnaeus. After 6-8 days, the signs of parasitism were quite evident. The first and second instars of white cabbage butterflies were recorded as the preferred hosts of wasps. The parasitoids completed their life cycle inside the host larva and emerged in an irregular mass of yellow-colored silken cocoons. They emerged from the cocoon after 7.3±1.3 days and were more than thirty-five individuals (38.6±3.05) in each mass of the cocoon (Fig. 7).

Identification of Parasitoids

The parasitoids were preserved in 95% ethanol until further use. After emergence, the parasitoids were identified as *Cotesia glomerata* by *Dr. Sarwan Kumar*, Senior Entomologist and Associate Professor, Punjab Agriculture University, Ludhiana, Punjab, India.

Discussion

The parent-host plant accessibility and quality influenced pest population dynamics by affecting both immature and adult functions. The influence of host plant on the fitness was observed not only in *P. brassicae* but also in other insects like *Helicoverpa armigera* (Hubner) and *Plutella xylostella* (Linnaeus).[24,28,30]

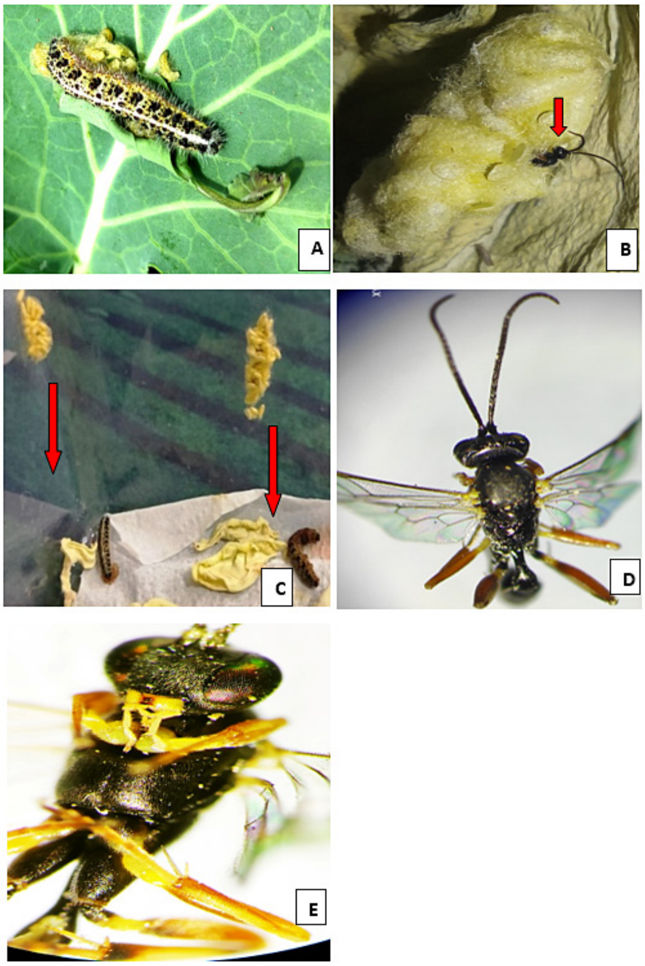

Fig. 7: Parasitoid of *Pieris brassicae* (a) Parasitoid emergence (b) Adult *Cotesia glomerata* emerging from cocoon (c) Mortality of parasitized larvae (d & e) Dorsal & ventral view of *Cotesia glomerata*

In the present study, the developmental period of *P. brassicae* varied with diverse hosts. For example, it was longest on *R. sativus* and shortest on *B. oleracea* var. *capitata.* This difference was due to diverse food sources used by the parents during the larval period. A similar presumption had been reported for *P. xylostella* reared on diverse *Brassica* crops including cabbage.[10] Besides, the host plants also influenced insect development rate.[32] Our results showed that the longest and shortest development times of individuals from neonate to the end of the pupal stage were recorded on radish and cabbage, respectively. Hence, a shorter development time and lower rate of mortality on a host was inductive of that particular host. This was in accordance with the study conducted by Awmack and Leather, 2002.[3] Therefore, it reinforced the hypothesis that cabbage is a more suitable host for the development of *P. brassicae* than cauliflower and radish. Besides, many factors that affected host suitability included its tissue texture and nutrient contents. In our study, the rate of early instar mortality was found to be higher on radish compared to cabbage and cauliflower due to the presence of hard tissue texture and trichome-like appendages in radish. This was supported by the studies of Gupta, 2002 and Ahmad et al. 2007.[11, 12] K value was a key factor primarily responsible for the increase or decrease in number from one generation to another. The total generation mortality, 'K' of *P. brassicae* was recorded maximum on radish (0.48) followed by cauliflower (0.26) and cabbage (0.15).

C. glomerata, an effective parasitoid of *P. brassicae*, was found in Palampur by Sood et al 2011.[29] The mortality of larvae at the end of the fifth instar had also been reported by Laing and Levin, 1982 after the major damage to the crop.[15, 18] In the field, *C. glomerata* had already been identified as a major natural enemy of *P. brassicae* and the host larvae expired 2 or 3 days later but did not feed throughout this time.[9] In Iran, *C. glomerata* was observed as a parasitoid of *P. brassicae*, whereas in Kashmir Valley, it was also observed as a parasitoid of the cabbage butterfly.[6, 23] No pupal parasitism was recorded in the study.

Conclusion

In conclusion, this study provides information on the fitness of *P. brassicae* on diverse host plants. Understanding how the quality of the *Brassica* host plant affects the life table parameters of *P. brassicae* helps in understanding population dynamics and selecting appropriate management approaches for this pest. Radish can be employed as a trap crop when cabbage and cauliflower are the major vegetable cash crops. Besides, prolonged developmental time can enhance the exposure of an insect to its natural enemies. Many beneficial insects have been and continue to be raised for release as biological pest control strategies. Knowledge of biology and natural enemies is required for pest management strategies that are compatible with IPM (Integrated Pest Management), and a successful management plan requires information about a species biology including its diet and life cycle. Biocontrol is a promising solution for both environmental protection and pest problems in agriculture. *C. glomerata* is a successful biocontrol agent that can be used as a principal parasitoid against *P. brassicae.* Conservation of this wasp species and relocation of unhatched wasp cocoons to contaminated sites can help in the natural control of the pest. The parasitoid species, which completes its developmental cycle on caterpillars of other species from the genus *Pieris* and *Pontia* (*P. rapae*, *P. protodice*, *P. napi*, and *Pontia daplidice*) has the potential to reduce the harmfulness of the caterpillars from the genus *Pieris*. Further research, particularly concerning parasitism of the egg and pupal stages, is likely to reveal more parasitoid species in Rajouri.

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Conflict of Interest

We have no conflicts of interest to disclose. All authors accept the previous version of the manuscript.

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